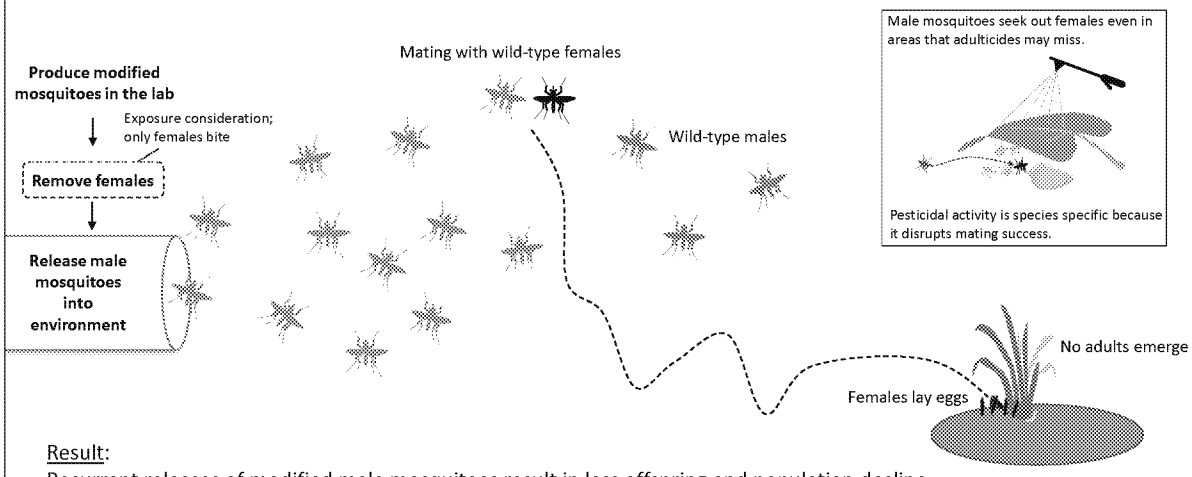
Emerging Mosquito Technologies: Concepts and Novel Considerations

Principles of emerging mosquito technologies

Concept: Introduce modified male mosquitoes into wild populations to achieve population suppression.



Result:
Recurrent releases of modified male mosquitoes result in less offspring and population decline.

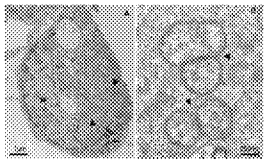
Current status of emerging mosquito products

- EPA has evaluated two types of emerging mosquito technologies for population suppression:
  1. Bacteria-infected mosquitoes.
  2. Genetically engineered mosquitoes.

- Developers have modified two mosquito species: Asian Tiger Mosquito (*Aedes albopictus)* and Yellow Fever Mosquito (*Aedes aegypti)*.
  - Both mosquito species are invasive to the U.S. and are responsible for transmitting diseases such as Zika, Dengue, and Chikungunya.

Wolbachia-infected mosquitoes

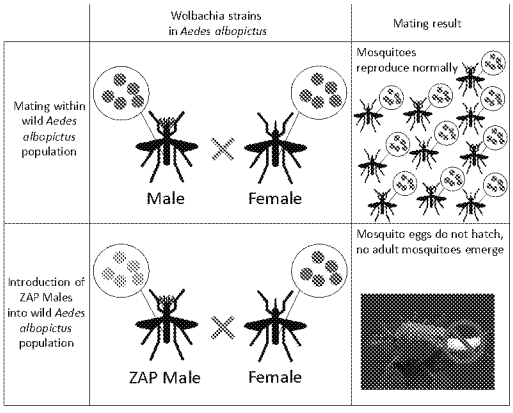
- MosquitoMate Inc., has received multiple EUPs and one registration for their *Wolbachia*-infected mosquitoes.
  - Partners with Verily Life Sciences (Google subsidiary).
- *Wolbachia* is a bacterium that naturally occurs in an estimated 65% of insects as well as some arthropods and crustaceans.
  - There are an estimated 5.5 million insect species.
- *Wolbachia* was introduced into *Aedes albopictus* and *Aedes aegypti* mosquitoes.



McMeniman *et al.*, 2008

Wolbachia-infected mosquitoes

- Females are mechanically separated from males in the lab.
- *Wolbachia*-infected males are released into environment to mate with wild-type females.
- Because the males carry a different strain of *Wolbachia* than the wild-type females, their offspring do not survive into adulthood.
- The introduced *Wolbachia* bacterium naturally occurs in a different mosquito species.
- Exposure to *Wolbachia*-infected mosquitoes is negligible.



Genetically-engineered mosquitoes

- Oxitec Ltd., developed *Aedes aegypti* mosquitoes (OX513A) that produce a protein that is lethal to their offspring.
- Oxitec applied for an EUP and a full registration for OX513A, but retracted both before completion of the full risk assessment.
  - EPA published a Notice of Receipt and received ~250,000 comments from the public.
- Oxitec's 2nd generation genetically engineered mosquito (OX5034) is currently under review for an EUP.
  - Some of the previously received public comments are still pertinent.

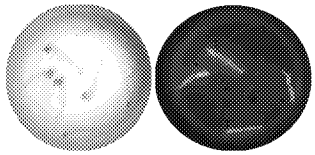
Oxitec OX5034 application (2nd generation)

- Requesting EUP for 2 years to assess efficacy in the United States.
- Releases proposed on 5,000 acres total in Harris County, Texas and Monroe County, Florida.
- Deficiencies were identified during the preliminary technical screen and a 10-day deficiency letter was sent to the company on July 1st (due July 16th).
- PRIA date: November 1, 2019.

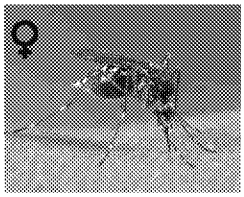
Principles of Oxitec's OX5034 technology

- Two novel proteins genetically engineered into *Aedes aegypti* mosquitoes (OX5034).
- tTAV and DsRed2.
  - tTAV active ingredient = kills female mosquitoes only.
  - DsRed2 inert = fluorescent marker protein.
- tTAV protein accumulates in juvenile females to lethal levels.
  - tTAV is processed differently in male mosquitoes.
- Females genetically (as opposed to mechanically) separated from males in the lab.
- Wild-type females lay eggs: Only male mosquitoes will emerge.
- Exposure to tTAV and DsRed2 is negligible <u>if</u> tTAV kills females 100% of the time.

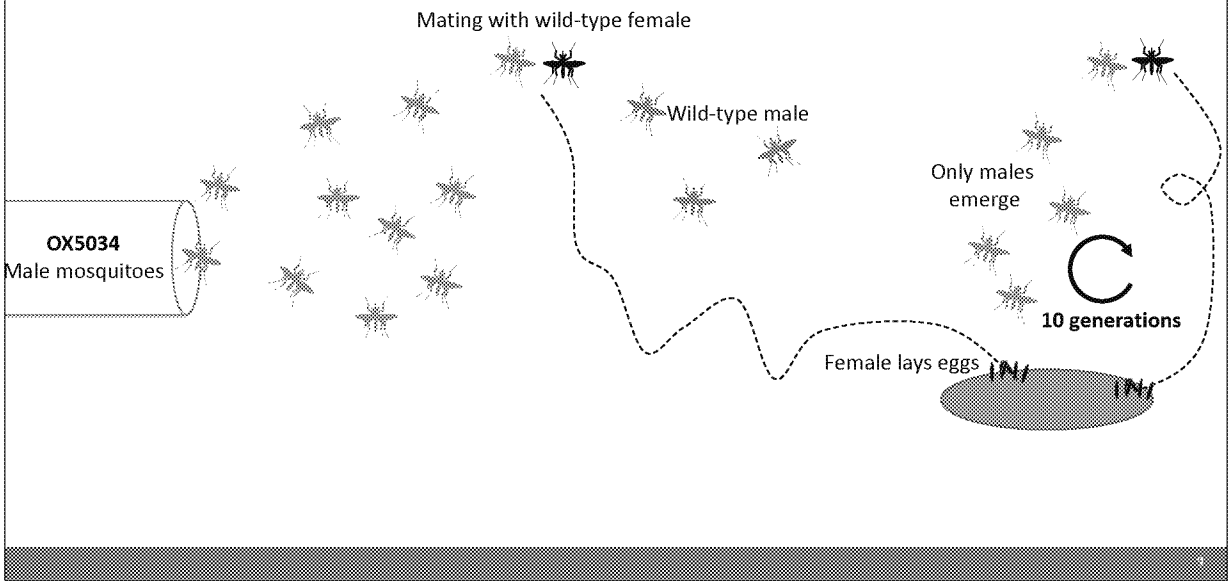
DsRed2 fluorescence in OX5034 larvae



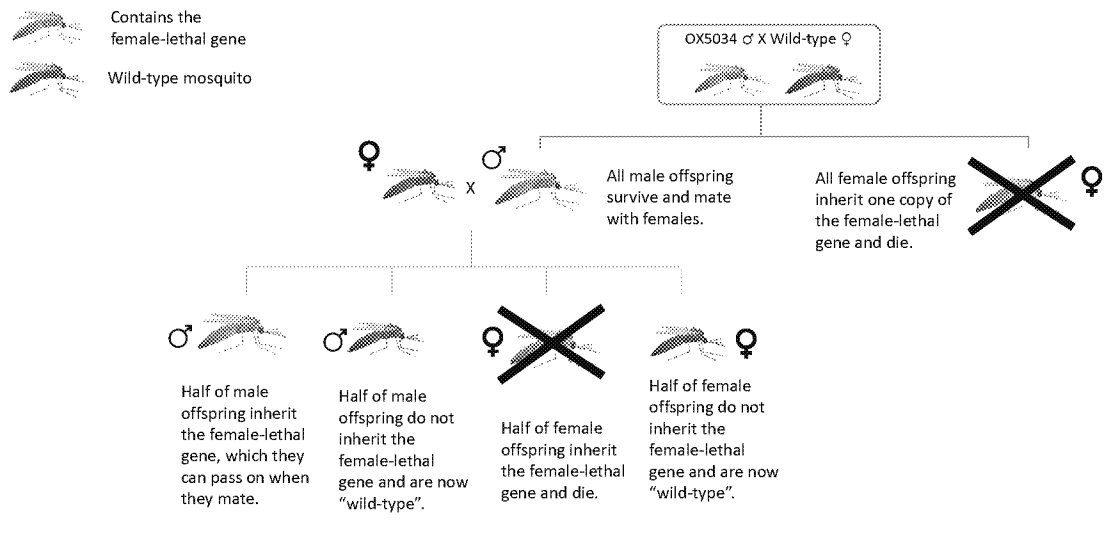
Only female mosquitoes bite



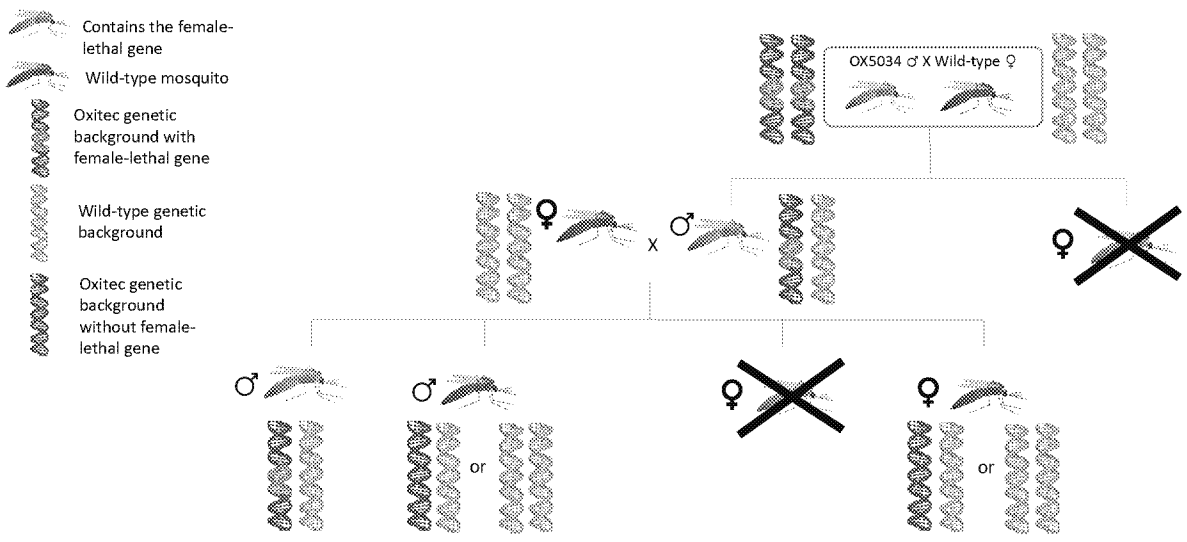
Principles of Oxitec's OX5034 technology



Residual effects of OX5034 across mosquito generations



OX5034 genetic background moves into the wild population

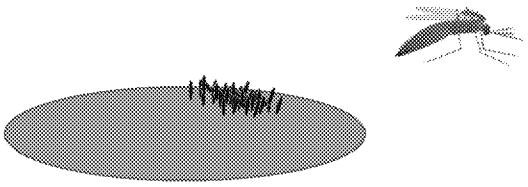


Uncertainties not addressed in application

Because some offspring of released mosquitoes survive and integrate into the wild population, which traits from the lab strain are important to characterize?

Example trait of interest:

Egg-laying ability



Key Deficiencies in EUP application

Proposed experimental design

- Oxitec requests 5,000 acres to test the efficacy of the OX5034 *Aedes aegypti* males.
- However, they propose to test multiple release rates, multiple release mechanisms, and multiple trial designs.
- Given the large number of variables, it is unclear if the requested 5,000 acres is sufficient. The number of treatments, replicates, and necessary acres must be clarified.

Key Deficiencies in EUP application

- Oxitec's safety argument hinges on no exposure to female GE mosquitoes.
- The data supporting the safety of the tTAV and DsRed2 proteins are not adequate and cannot be satisfied in the short term.
- DsRed2 has an unresolved risk concern previously identified for OX513A: DsRed2 is similar to a protein reported in the scientific literature to be a potential allergen.
- OPP requested data to determine whether tTAV will kill all female offspring. If not all females die, then exposure to tTAV and DsRed2 may be possible through transfer of the proteins through the saliva of biting females.

How EPA identifies potential allergenic proteins

- EPA evaluates the potential for novel proteins to be allergenic by comparing them (their amino acid sequence) against proteins known to, or suspected to be, allergens using internationally-recognized standards (CODEX Alimentarius Guidelines, 2003).
- Two publicly-available databases: AllergenOnline (Univ. of Nebraska) and COMPARE (ILSI/HESI).

Evaluation of DsRed2 in OX513A (1st generation)

- OPP's technical screen revealed that the amino acid sequence of DsRed2 produced in the GE mosquito is substantially similar to "Akane", a coral protein.
- Both AllergenOnline and COMPARE databases listed Akane as a potential allergen based on a report in the public literature. The researchers attributed allergic reactions observed in fishermen to the coral Akane protein.
- In response to requests from Oxitec, both panels reevaluated the research publication and subsequently removed Akane from their databases.
- Both panels independently agreed that the researchers failed to provide enough evidence to demonstrate that Akane was the cause of the allergic reactions.
- For OPP, removal of Akane from the two databases does not mean that the coral protein, and thus DsRed2, is not an allergen. Additional product data is needed to make that determination.

Key Deficiencies in EUP application

Human health risk assessment for OX5034

- OPP intends on relying on a no exposure argument.
  - i.e., tTAV will kill 100% of female mosquitoes in the offspring.
- However, if female mosquitoes with the trait are present in the environment, people will be bit and could be exposed to tTAV and dsRed2.

Information needed from Oxitec

- Mate Oxitec's OX5034 males with different wild-type *Aedes aegypti* strains, e.g., wild-type females from Florida, and record lethality of the tTAV trait in the resulting offspring.

Considerations for OPP's risk communication

- If Oxitec provides evidence that no OX5034 females survive, then exposure to biting females will be negligible. This would allow OPP to make a safety determination under FIFRA.
- In the Court of Public Opinion any release of GE female biting mosquitoes is a risk communication problem, given the public's concerns with genetic engineering.
- The "no exposure" argument may satisfy the Court of Public Opinion.
- However, as presented at the recent NPIC briefing on risk communication, the public is less concerned with risk for which they make the decision than for risk in which they have no decision making role, i.e., voluntary vs. involuntary.